

SKEIKY *et al.*
Application No.: 09/688,672
Page 2

In the Specification:

Please replace the paragraph beginning at page 4, line 2, with the following:

B¹
--Figure 1 shows the nucleic acid sequence of a vector encoding TbF14 (SEQ ID NO:91). Nucleotides 5096 to 8594 encode TbF14 (SEQ ID NO:51). Nucleotides 5072 to 5095 encode the eight amino acid His tag (SEQ ID NO:92); nucleotides 5096 to 7315 encode the MTb81 antigen (SEQ ID NO:1); and nucleotides 7316 to 8594 encode the Mo2 antigen (SEQ ID NO:3).--

Please replace the paragraph beginning at page 4, line 10, with the following:

B²
--Figure 2 shows the nucleic acid sequence of a vector encoding TbF15 (SEQ ID NO:92). Nucleotides 5096 to 8023 encode the TbF15 fusion protein (SEQ ID NO:53). Nucleotides 5072 to 5095 encode the eight amino acid His tag region; nucleotides 5096 to 5293 encode the Ra3 antigen (SEQ ID NO:5); nucleotides 5294 to 6346 encode the 38 kD antigen (SEQ ID NO:7); nucleotides 6347 to 6643 encode the 38-1 antigen (SEQ ID NO:9); and nucleotides 6644 to 8023 encode the FL TbH4 antigen (SEQ ID NO:11).--

Please replace the paragraph beginning at page 4, line 21, with the following:

B³
--Figure 7 shows the nucleic acid and predicted amino acid sequences of three fragments of HTCC#1. (a) and (b) show the sequences of two overlapping fragments: an amino terminal half fragment (SEQ ID NOS:15 and 16) (residues 1 to 232 223), comprising the first trans-membrane domain (a) and a carboxy terminal half fragment (SEQ ID NOS:17 and 18) (residues 184 to 392), comprising the last two trans-membrane domains (b); (c) shows a truncated amino-terminal half fragment (SEQ ID NOS:19 and 20) (residues 1 to 129 128) devoid of the trans-membrane domain.--

Please replace the paragraph beginning at page 4, line 29, with the following:

B4
--Figure 9a shows the nucleic acid and predicted amino acid sequences of a recombinant HTCC#1 lacking the first trans-membrane domain (SEQ ID NOS:21 and 22) (deleted of the amino acid residues 150 to 160). Figure 9b (SEQ ID NOS:201 and 202) shows the nucleic acid and predicted amino acid sequences of 30 overlapping peptides (SEQ ID NOS:94-123 and 124-153, respectively) of HTCC#1 used for the T-cell epitope mapping. Figure 9c illustrates the results of the T-cell epitope mapping of HTCC#1. Figure 9d shows the nucleic acid and predicted amino acid sequences of a deletion construct of HTCC#1 lacking all the trans-membrane domains (SEQ ID NOS:23 and 24) (deletion of amino acid residues 101 to 203)--

Please replace the paragraph beginning at page 5, line 20, with the following:

B5
--Figure 17 shows the amino acid sequence of *Mycobacterium tuberculosis* the secreted form of antigen DPPD (SEQ ID NO:154).--

Please replace the paragraph beginning at page 6, line 7, with the following:

B6
--SEQ ID NO:15 is the nucleic acid sequence of an amino terminal half fragment (residues 1 to 232) of HTCC#1, comprising the first trans-membrane domain.--

Please replace the paragraph beginning at page 6, line 9, with the following:

B7
--SEQ ID NO:16 is the predicted amino acid sequence of an amino terminal half fragment (residues 1 to 232) of HTCC#1.--

Please replace the paragraph beginning at page 6, line 16, with the following:

B⁸ --SEQ ID NO:19 is the nucleic acid sequence of a truncated amino-terminal half fragment (residues 1 to 129) of HTCC#1 devoid of the trans-membrane domain--

Please replace the paragraph beginning at page 6, line 18, with the following:

B⁹ --SEQ ID NO:20 is the predicted amino acid sequence of a truncated amino-terminal half fragment (residues 1 to 129) of HTCC#1--

Please replace the paragraph beginning at page 7, line 8, with the following:

B¹⁰ --SEQ ID NO:33 is the nucleic acid sequence encoding the MTI (Mtb9.9A) antigen. SEQ ID NO:155 is a second nucleic acid sequence encoding the MTI (Mtb9.9A) antigen--

Please replace the paragraph beginning at page 7, line 10, with the following:

B¹¹ --SEQ ID NO:34 is the amino acid sequence of the MTI antigen. Mtb9.9A (MTI-A) ORF peptides are given in SEQ ID NOS:156-171--

Please replace the paragraph beginning at page 7, line 13, with the following:

B¹² --SEQ ID NO:36 is the amino acid sequence of the MSL antigen. Mtb9.8 (MSL) ORF peptides are given in SEQ ID NOS:172-186--

Please replace the paragraph beginning at page 72, line 17, with the following:

B 13
--TbF14 is a fusion protein of the amino acid sequence encoding the MTb81 antigen fused to the amino acid sequence encoding the Mo2 antigen. A sequence encoding Mo2 was PCR amplified with the following primers: PDM-294 (T_m 64°C) CGTAATCACGTGCAGAAGTACGGCGGATC (SEQ ID NO:187) and PDM-295 (T_m 63°C) CCGACTAGAATTCACCTATTGACAGGCCCATC (SEQ ID NO:188).--

Please replace the paragraph beginning at page 72, line 27, with the following:

B 14
--A sequence encoding MTb81 was PCR amplified with the following primers: PDM-268 (T_m 66°C) CTAAGTAGTACTGATCGCGTGTCTGGTGGGC (SEQ ID NO:189) and PDM-296 (T_m 64°C) CATCGATAGGCCTGGCCGCATCGTCACC (SEQ ID NO:190). The amplification reaction was performed using the same mix as above, as follows: denaturation at 96°C for 2 min; followed by 40 cycles of 96°C for 20 sec, 65°C for 15 sec, 72°C for 5 min; and finally by 72°C for 5 min.--

Please replace the paragraph beginning at page 73, line 12, with the following:

B 15
--TbF6 was made as follows (see PCT/US99/03268 and PCT/US99/03265). First, the FL (full-length) TbH4 coding region was PCR amplified with the following primers: PDM-157 CTAGTTAGTACTCAGTCGCAGACCGTG (SEQ ID NO:191) (T_m 61°C) and PDM-160 GCAGTGACGAATTCACCTTCGACTCC (SEQ ID NO:192) (T_m 59°C), using the following conditions: 10 μ l 10X Pfu buffer, 1 μ l 10 mM dNTPs, 2 μ l 10 μ M each oligo, 82 μ l sterile water, 1.5 μ l Accuzyme (ISC, Kaysville, UT), 200 ng *Mycobacterium tuberculosis* genomic DNA. Denaturation at 96°C was performed for 2 minutes; followed by 40 cycles of 96°C for 20 seconds, 61°C 15 seconds, and 72°C 5 minutes; and finally by 72°C 10 minutes.--

Please replace the paragraph beginning at page 73, line 23, with the following:

B¹⁶
-pET28Ra3/38kD/38-1A was made by inserting a *Dra*I site at the end of 38-1 before the stop codon using the following conditions. The 38-1 coding region was PCR amplified with the following primers: PDM-69
GGATCCAGCGCTGAGATGAAGACCGATGCCGCT (SEQ ID NO:193) (T_m 68°C) and PDM-83 GGATATCTGCAGAATTCAGGTTTAAAGCCCATTGCGA (SEQ ID NO:194) (T_m 64°C), using the following conditions: 10 μ l 10X Pfu buffer, 1 μ l 10 mM dNTPs, 2 μ l 10 μ M each oligo, 82 μ l sterile water, 1.5 μ l Accuzyme (ISC, Kaysville, UT), 50 ng plasmid DNA. Denaturation at 96°C was performed for 2 minutes; followed by forty cycles of 96°C for 20 seconds, 66°C for 15 seconds and 72°C for 1 minute 10 seconds; and finally 72°C 4 minutes--

Please replace the paragraph beginning at page 74, line 7, with the following:

B¹⁷
-Fusion construct TbF10, which replaces the N-terminal cysteine of 38 kD, was made as follows. To replace the cysteine residue at the N-terminus, the 38kD-38-1 coding region from the TbF fusion (described in WO/9816646 and WO/9816645) was amplified using the following primers: PDM-192
TGTGGCTCGAAACCACCGAGCGGTTC (SEQ ID NO:195) (T_m 64°C) and PDM-60
GAGAGAATTCTCAGAAGCCCATTGCGAGGACA (SEQ ID NO:196) (T_m 64°C), using the following conditions: 10 μ l 10X Pfu buffer, 1 μ l 10 mM dNTPs, 2 μ l 10 μ M each oligo, 83 μ l sterile water, 1.5 μ l Pfu DNA polymerase (Stratagene, La Jolla, CA), and 50 ng plasmid TbF DNA. The amplification reaction was performed as follows: 96°C for 2 minutes; followed by 40 cycles of 96°C for 20 seconds, 64°C 15 seconds, and 72°C 4 minutes; and finally 72°C 4 minutes. Digest the PCR product with *Eco* RI and clone into pT7 Δ L2Ra3 which has been digested with *Stu* I and *Eco* RI. Digest the resulting construct with *Nde* I and *Eco*RI and clone into pET28 at those sites. The

resulting clone (called TbF10) will be TBF + a cysteine at the 5' end of the 38kD coding region. Transform into BL21 and HMS 174 with pLys S

Please replace the paragraph beginning at page 77, line 14, with the following:

B 18
One of the identified positive wells was further broken down until a single reactive clone (HTCC#1) was identified. Sequencing of the DNA insert followed by search of the Genebank database revealed a 100% identity to sequences within the *M. tuberculosis* locus MTCY7H7B (gene identification MTCY07H7B.06) located on region B of the cosmid clone SCY07H7. The entire open reading frame is ~1,200 bp long and codes for a 40 kDa (392 amino acids) protein (Fig. 1; HTCC#1 FL). Oligonucleotide PCR primers [5' (5'-CAA TTA CAT ATG CAT CAC CAT CAC CAT CAC ATG AGC AGA GCG TTC ATC ATC-3'; SEQ ID NO:197) and 3' (5'-CAT GGA ATT CGC CGT TAG ACG ACG TTT CGT A-3'; SEQ ID NO:198)] were designed to amplify the full-length sequence of HTCC#1 from genomic DNA of the virulent Erdman strain.

Please replace the paragraph beginning at page 78, line 27, with the following:

B 19
Specifically, the C-terminal fragment of antigen MTB32A was amplified by standard PCR methods using the oligonucleotide primers 5' (CAA TTA CAT ATG CAT CAC CAT CAC CAT CAC ACG GCC GCG TCC GAT AAC TTC; SEQ ID NO:199) and 3' (5'-CTA ATC GAA TTC GGC CGG GGG TCC CTC GGC CAA; SEQ ID NO:200). The 450 bp product was digested with NdeI and EcoRI and cloned into the pET17b expression vector similarly digested with the same enzymes.

Please cancel the informal "SEQUENCE LISTING", pages 1-26, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 119, at the end of the application.